

THE EARLY DEVELOPMENT OF A POLYCLAD, *PLANOCERA INQUILINA* Wh.

BY FRANK M. SURFACE.

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It gives me great pleasure to express my deep indebtedness to Prof. E. G. Conklin for his many suggestions and kind encouragement throughout this and other work. I also wish to acknowledge the many favors I have received from the University of Pennsylvania by which this work was made possible. My thanks are also tendered to the donor of the University of Pennsylvania room at the Marine Biological Laboratory at Woods Holl during the summer of 1906.

INTRODUCTION.

Our knowledge of the development of the polyclad worms is based chiefly on the admirable works of Arnold Lang. In his extensive monograph of this class of the Turbellaria, published in 1884, Lang has not only summarized the results of all previous investigators, but has himself added very materially to our knowledge of their embryology.

Previous students of polyclad embryology have shown the undoubtedly spiral nature of the cleavage up until a late stage of segmentation. In this respect the development of these platodes corresponds closely with the cleavage of annelidan and molluscan eggs. Wilson (92) says (p. 439): "Up to a late stage in the spiral period (twenty-eight cells) every individual blastomere and every cell division is represented by a corresponding blastomere and a corresponding cell division in the embryo of the polyclad and in that of the gasteropod." The same practically may be said of the annelid.

This striking resemblance in the early ontogeny of three great groups of animals should not be without some significance. Yet, according to the accounts of these earlier investigators, the later history of the cells in the polyclad embryo differs very greatly from that of the apparently homologous cells in the annelids and mollusks. The difference is so marked that Conklin (97) has characterized it as "very great, perhaps irreconcilable." Wilson (92) has summarized this difference as follows (p. 441): "In the polyclad the first group of micromeres gives rise to the entire ectoblast, the second and third groups to the mesoblast, the macromeres to the entoblast. In the mollusk and annelid, on the other hand, the second and third groups of micromeres give rise to the ectoblast, like the first set, and the mesoderm arises subsequently."

The formation of the ectoderm from the first quartet alone and of the mesoderm from the whole of the second and third quartets has been a serious stumbling block to those embryologists who have attempted to establish cellular homologies. Wilson in a later paper (94) has cited this case as the climax in the contradictions of comparative embryology. A number of recent writers have expressed a doubt as to the correctness of Lang's interpretations. Thus Mead (97) writes (p. 289): "I am not convinced that the cells described by Lang do give rise to the mesoderm, and I believe it possible that the mesoderm of the polyclad is formed in the same manner and from exactly the same cell as in the annelids with unequal cleavage."

This was the state of affairs until 1898 when Prof. Wilson published in his paper on "Cell Lineage and Ancestral Reminiscences" some observations on a Pacific coast species of *Leptoplana*. Wilson's investigations showed that Lang was wrong in certain particulars, but did not fulfill Mead's prediction with regard to the mesoblast. Wilson found that each of the first three quartets of micromeres contributed to the formation of ectoderm, but also found that the mesoderm arose by inbudding from cells of the second quartet and possibly some from

the third. Wilson's work was not a detailed study of the development, and it leaves the impression that there may be something still undiscovered in the embryology of these interesting worms. Most of Wilson's work and all of Lang's was on the living egg. Every student of embryology knows how unsatisfactory this method is for the late stages of segmentation, unless checked by properly fixed and stained material.

In view of these facts it has seemed worth while to enter into a more or less detailed account of the cell lineage of this form and gain, if possible, data to support or refute the theory of cellular homology. Besides, such data should throw some light on the phylogeny of this class of the Turbellaria.

Lang has given so complete a review of the literature on polyclad embryology previous to 1884 that it would be mere repetition to go over that in detail here. The earliest investigators were Girard (1846-1854), Vaillant (1866-1868) and Keferstein (1868). Of these the work of Keferstein was by far the best. Three other investigators had studied polyclad development previous to 1884. The first of these was Hallez (1878-1879). Hallez recognized but one quartet of micromeres. From this the ectoderm arose. The macromeres budded off four small cells at the oral pole which he believed formed the mesoderm. He described four other later buds at the oral pole which formed the wall of the gut. The work of Goette (1878-1882) and Selenka (1881) followed close on that of Hallez. Goette also observed but one quartet of micromeres in *Stylochus pillidium*. When the ectoderm had reached the equator of the egg two to four small cells were formed at the oral pole (lower endoderm). Later the large macromeres budded large cells towards the aboral pole. These cells formed the upper endoderm. From the upper and lower endoderm the wall of the alimentary canal was formed, while the large macromeres became food yolk. Goette found no mesoderm.

Selenka (1881) determined that two quartets were given off. According to him the first formed ectoderm and the second the mesoderm. Four small cells were formed at the oral pole (lower endoderm of Goette), from which he believed the entire wall of the alimentary canal was formed. He found no upper endoderm.

Lang (1884) found that three quartets of micromeres were formed. As already stated he believed the first formed the ectoderm, the second and third the mesoderm. From the large macromeres four small cells were formed at the oral pole (lower endoderm). Then each of the large macromeres (fourth quartet) budded towards the aboral pole an upper

endoderm cell, as Goette had found. He derived the alimentary canal from the upper and lower endoderm cells, while the macromeres (middle endoderm) broke up into food yolk and were absorbed by the other cells. All of the last four writers found that the posterior macromere behaved differently from the other three in that it divided with its nuclear spindle lying horizontally, thus giving rise to five macromeres (fourth quartet).

MATERIAL AND METHODS.

The following paper is based upon the study of a species of polyclad, *Planocera inquilina*, described by Prof. Wheeler in 1894. The material was obtained at the Marine Biological Laboratory at Woods Hole, Massachusetts, during the months of July and August of 1906.

Planocera inquilina is peculiar among polyclads in that it leads an apparently parasitic life. These worms are found in the branchial chamber of the large whelk, *Sycotypus canaliculatus* Gill. As Wheeler suggests, it seems probable that they live on the excretory or waste products of this gasteropod. No evidences that they feed on the tissues of the host have been found. The adult worms were obtained in considerable abundance, averaging about three worms for every whelk opened. The adult polyclads were transferred to dishes of sea water, in which the water was changed by means of a system of balanced syphons. These syphons served to keep the water free from sand and dirt, and also prevented the overflow of the water and the escape of the worms. The animals usually laid eggs soon after being brought into the laboratory. As described by Wheeler, the eggs are laid in spiral, gelatinous capsules containing anywhere from 100 to 2,000 eggs each. Each egg is surrounded by a separate egg membrane and the whole is imbedded in the capsule material. In many cases two eggs are deposited in a single egg membrane, both of which develop normally. This is the usual way in which polyclad eggs are deposited. The tough capsule is difficult to penetrate with fixing and staining reagents. This fact no doubt is one of the chief reasons why so few embryologists have worked on polyclad development. The eggs of *Planocera inquilina* seem more favorable in this respect, and with care it is possible to get very good preparations. The egg capsules were deposited against the sides of the dishes, and it was necessary to cut them away with a scalpel.

Wheeler (94) did not succeed in getting the eggs of this species to develop under laboratory conditions. On the contrary I experienced no difficulty of this kind. Stages from the maturation to the free

swimming, Müller's larvæ were obtained without difficulty. I do not know wherein my methods differed from Wheeler's. He suggested that the water in the laboratory was too warm. However I made no attempt to keep it cool, and in some cases the sun shone directly on the dishes without apparently affecting the eggs. The adult animals however would live for only a few days. After the first day they became very sluggish and their bodies began to break up in a manner similar to that described by Wheeler.

Although I studied the question a good deal, I have never been able to ascertain where the eggs of this polyclad are laid under natural conditions. The animals were laying throughout the entire summer from June to September, yet I have never found a single capsule except when deposited in my dishes. I have repeatedly searched the interior of the branchial chamber of the whelks in which adult worms were found, but to no avail. I have also examined carefully the shells of these gasteropods, both inside and out, but no evidence of egg capsules was found. I found that the worms always laid soon after being removed from the whelk to the dishes of sea water, and it is possible that this is the normal stimulus to egg deposition. If such is the case the adult animals must deposit their eggs on stones or other smooth objects on the bottom. In such a case both adult and young would have to run the risk of again finding a *Sycotypus* and entering its branchial chamber. The risk seems to be considerable, and the number of eggs deposited by an individual is perhaps hardly sufficient to warrant such an hypothesis.

The early divisions up to the forty-eight- or fifty-cell stage were followed and figured in the living egg. The eggs are rather opaque and it is difficult to be certain concerning many of the divisions. This whole portion of the cell lineage was later gone over in the stained preparations and the previous observations on the living material were verified or corrected.

Eggs were fixed in various solutions, among which were sublimate acetic both aqueous and in 95 per cent. alcohol, Gilson's murcuro-nitric, picro-sulphuric, picro-acetic, Perenney's fluid and Flemming's solution. Of these the sublimate-acetic mixtures and Gilson's fluid proved most valuable. For staining whole mounts Conklin's (02) picro-hæmatoxlyn was used. Slightly stronger hæmatoxlyn than recommended by Conklin was found better for these particular eggs. The eggs were then cleared in xylol and mounted in balsam. It was impossible on account of their small size to remove the eggs from the capsules, but it was found that they cleared better if the capsules were torn into small pieces.

In studying the cell lineage the chief difficulty experienced was in not being able to rotate the eggs under the cover glass. The eggs are not orientated in any definite direction within the capsules, and it was necessary to pick out for study those eggs which were favorably oriented. Besides it is particularly difficult to determine the lineage of certain cells if one is able to view them from one side only. The fact that the eggs could not be rotated accounts for some of the drawings being from a somewhat oblique view.

The results obtained from studying the whole mounts were checked as far as possible by the use of serial sections. It was found necessary to bleach the Flemming material with peroxide of hydrogen before sectioning. A number of stains were used for the sections, but Delafield's hæmatoxlyn, either in *toto* or on the slide, proved most useful. A combination of thionin and acid fuchsin also gave good results. There is too much yolk in these eggs to use Haidenhain's iron-alum-hæmatoxlyn to advantage.

NOMENCLATURE.

The system of nomenclature followed in the cell lineage of this paper is that used by Chabry (87), Wilson (92) and Conklin (97), with slight modifications. This system is the same as has been used by Child (1900), Treadwell (1901), Casteel (1904), Nelson (1904), and many others. For the sake of convenience the chief points are repeated here. Each of the four quadrants of the egg is denoted by one of the first four letters of the alphabet. The left quadrant is *A*, the anterior *B*, the right *C*, and the posterior *D*. The four macromeres form the basal quartet; the first group of four micromeres to be separated from these is the first quartet, and so on. A micromere is denoted by a lower case letter, while the capital letters are reserved for the corresponding macromeres. The number of the quartet to which a micromere belongs is indicated by a coefficient, while the cell generation is shown by the exponent. Of the two cells of any division of a micromere (except *4d*), the one lying nearer the animal pole is regarded as the stem cell and receives the smaller exponent. Thus $2a^1$, a cell of the second quartet in the *A* quadrant, will divide into $2a^{1.1}$ and $2a^{1.2}$. $2a^{1.1}$ lies nearer the animal pole than $2a^{1.2}$. $2a^{1.1}$ will divide into $2a^{1.1.1}$ and $2a^{1.1.2}$. In the case of the divisions of the mesentoblast, *4d*, the lower cell is regarded as the stem cell and receives the smaller exponent (Conklin, 97).

A division is to the right, dextrotropic, if the upper cell lies to the right of the lower when viewed by an imaginary observer situated at

the animal pole and facing the cell in question. If the upper cell is to the left of the lower the division is keotropic (Lillie, 95). If the spindle is horizontal, *i.e.*, the cleavage meridional, the cell to the right receives the smaller exponent.

Following Child (1900), the macromeres receive the coefficient of the quartet to which they last contributed. Thus 3A gave rise at its last division to 3a. Further details of the system will become evident by reference to the tables of cell lineage and to the figures.

THE LIVING EGG.

The living egg of *Planocera inquilina* consists of a uniformly dense mass of granules which vary only slightly in size. Between these granules is a light colored fluid substance. When the one-celled egg is strongly centrifuged for some time the yolk granules are compacted to one side and a cap of the light colored fluid, in which are only a few granules, lies at the opposite side. This cap of fluid occupies perhaps one-fourth or a little less of the entire egg. When the egg is crushed under a cover glass and examined with an immersion lens, minute bodies (microsomes?) are found in the fluid portion. These small bodies exhibit a constant "brownian" movement. When the egg is entire, however, no motion of any kind can be discerned.

The eggs of this species of Polyclad appear perfectly uniform throughout. Selenka, Goette and Lang have found that in many polyclad eggs there is a darker inner portion and a lighter outer layer to the eggs. I could make out no such differentiation in these eggs. Lang did not find this separation of substance in *Discocalis tigrina*.

Considerable time was spent during the summer attempting to experiment on these eggs. But in all cases where the conditions were varied from the normal the egg died in a short time and no results were obtained.

All the early cleavages as well as the maturation divisions occur at intervals of about one hour. The whole development proceeds rather more rapidly than in most polyclads. At the end of the second day or at the beginning of the third the embryo is completely covered by the small ectodermal cells. Cilia soon begin to form on these and by the third day the embryo begins to slowly rotate within the capsule. During the next day or two the cilia become better developed and the embryo rotates faster and faster. The rotation takes place first in one direction and then after a short pause in another. Occasionally they cease moving for some time. The eye spots appear about the fourth day. During the fourth and fifth days a number of homogeneous

yolk spherules can be seen inside the embryo. By the end of the fifth day the ciliated processes characteristic of the Müller's larvæ begin to appear and the embryo exhibits frequent contractions of its body. On the sixth day the larvæ begin to burst through the egg membranes and to swim about as typical Müller's larvæ. I did not succeed in keeping these larvæ more than two or three days, during which time they seemed to undergo but little change.

GENERAL ACCOUNT OF THE EMBRYOLOGY.

Following the example of many writers on embryology, it seems that the later detailed account can be made briefer and more readily understood if it is prefaced by a brief general sketch of the development. The segmentation of the egg is total and slightly unequal. From the first two divisions four cells result, of which one, the posterior, is slightly the largest. Three quartets of micromeres are then given off in alternating dextrotropic and laetotropic directions. The large cells of the basal quartet then bud off at their lower, vegetative pole four very small cells, which are to be regarded as the macromeres. The large upper cells of this division form the fourth quartet. The large posterior cell of this quartet, $4d$, behaves very differently in its future divisions from the other three. We may designate this cell as the "mesentoblast," following Conklin's nomenclature. At the stage with forty-four cells this mesentoblast buds into the interior of the egg a large cell, $4d^2$. Both of the mesentoblast cells then divide. In these divisions the nuclear spindles lie horizontally. From the lower pair of cells the greater part of the alimentary canal is derived. The upper pair probably contribute a small amount to the alimentary canal, while the larger portion goes to form the mesoderm of the body.

In the later development the chief axis of the egg, *i.e.*, the axis from the animal to the vegetative pole, becomes bent, so that the animal pole comes to lie at the anterior end of the embryo.

From the first quartet arises the ectoderm, covering the anterior and dorsal portions of the body. From cells of this quartet four strings of cells bud into the interior of the embryo and form the ganglion. The eyes arise in ectodermal cells of this quartet. The second quartet gives rise to the larger portion of the ectoderm on the ventral and posterior regions of the body. From cells of this quartet is formed most of the ectodermal pharynx. A portion of the second quartet is budded into the embryo and forms mesoderm. From this source arises probably only that mesoderm found around the blastopore and which is later concerned in the structures of the pharynx.

The third quartet consists of small cells from which apparently only ectoderm is derived. The individual divisions of these cells have not been traced very far, but there is every reason to believe that they form ectoderm only.

The history of the fourth quartet is peculiar. As already stated, the posterior cell 4*d* is the mesentoblast, from which the alimentary canal and a portion of the mesoderm arises. The other three cells of the fourth quartet, 4*a*, 4*b* and 4*c*, do not divide as long as their history can be traced. They, however, break up into a large number of homogeneous yolk spheres which are absorbed by the endoderm cells. The large nuclei of these three cells can be traced until the alimentary canal is partly formed.

The nuclei of the small macromeres show evidences of degeneration. These do not divide as long as they can be followed. They are carried into the embryo by the pharyngeal invagination of ectoderm, and it seems probable that they degenerate without giving rise to any morphological structure.

THE UNSEGMENTED EGG.

The unsegmented egg of *Planocera inquilina* is nearly spherical in shape and measures about one-tenth of a millimeter in diameter. In many cases, however, the eggs are pressed out of their normal shape by crowding within the capsule. The eggs when laid possess a large germinal vesicle which lies slightly to one side of the centre (fig. 1). This statement is not remarkable in itself, were it not for the fact that the eggs in the uterus of the adult possess a well-developed spindle with equatorial plate, centrosomes, etc. Apparently this spindle never goes farther than the equatorial plate stage and then degenerates so that the egg when deposited possesses a germinal vesicle. This phenomenon was first observed in certain polyclad eggs by Selenka (81*d*), and later Wheeler (94) has recorded it for this species. Gardiner (99) has studied this phenomenon in *Polycherus candatus* and concludes that the uterine spindle is due to abnormal conditions of the adult. I have not attempted to study this phenomenon in detail, but a casual survey shows that in animals which were fixed as soon as possible after removal from the whelk this uterine spindle was well developed. Since other animals from these same lots laid eggs which developed normally, one must conclude that if it is not a normal phenomenon it at least does not interfere with the later development. Selenka (81*d*) suggests that this spindle is of use in bringing the yolk granules to the centre of the egg, but, as Wheeler has noted, such could hardly be

the case here, since the distribution of the yolk granules is uniform throughout the egg.

Wheeler (94) has stated that the impregnation is probably what Whitman (91) has called "hypodermic." I have several times observed animals apparently in copulation. In this act the two animals remain in contact for some time and move about together. Most frequently the ventral sides of the animals were in contact. Fertilization is necessarily internal, although the means by which the sperm reach the eggs is not known.

Two maturation divisions occur after the egg is deposited. The first occurs about one hour after deposition and the second about one hour later. At each of these divisions the egg goes through some of the most remarkable contortions (fig. 2). The egg becomes very irregular, and processes occur from all sides. These processes consist of the more fluid substance and contain few yolk granules. In many cases parts of the egg are cut off entirely. In one case observed, the egg was actually cut into two, so that I first mistook it for a two-cell stage, but later these parts fused and it then underwent normal development. In some cases minute pieces seem to be cut off which do not fuse with the egg, for some time at least, but continue to float about between it and the egg membrane.

The movement of the egg substance is very slow. It takes about twenty minutes for an egg to pass through such a contortion and regain its normal spherical shape. The phenomenon is the same at each of the two maturation divisions. Similar phenomena have been observed by Hallez (79), Goette (82) and Selenka (81) in other polyclad eggs. Such amoeboid movements are also quite common in the eggs of other animals, especially annelids.

THE FIRST CLEAVAGE.

About one hour after the second maturation division the first cleavage furrow makes its appearance. The spindle for the first cleavage lies near the centre of the egg. The first two blastomeres are not of equal size, although the difference is slight (fig. 4). In order to make certain that there is a recognizable difference, I have made a number of camera drawings both of living and stained eggs. In all cases where it was not evident that the egg was pressed out of shape in its membrane the difference in size is quite easily recognized.

According to Lang (84) this difference in size of the first two blastomeres is very constant in polyclads. Lang says (p. 330): "Ich habe diese allerdings wenig auffallende Verschiedenheit in der Grösse

der zwei ersten Blastomeren, die Selenka bei Thysanozoon und Eurylepta constatirte, nicht nur bei *Discocelis tigrina*, sondern auch bei allen Pseudoceriden und Eurylepta nachweisen können. Ich glaube, dass sie auch bei allen Leptoplaniden existirt, obsehon sie hier schwer nachweisbar ist."

Since the polar bodies do not remain attached to the animal pole, I have been unable to ascertain whether there is a rotation of the spheres, indicating that the first cleavage is spiral, as Conklin (97) has shown for *Crepidula*. With the separation of the two cells their outlines become more or less irregular. Especially along their line of contact delicate protoplasmic processes extend outward. This is very much less marked than in the case of the maturation divisions, but there seems little doubt but that it is due to the same internal causes, whatever those may be. The same phenomenon may also be observed in several of the next succeeding cleavages, *i.e.*, at the four- or even eight-cell stage. But with each successive cleavage the processes are smaller and more delicate and the phenomenon less marked.

THE SECOND CLEAVAGE—TWO TO FOUR CELLS.

A little less than an hour after the first two cells have separated a furrow appears in each. These furrows often appear simultaneously, but in many cases one cell begins to divide in advance of the other. Lang finds that in *Discocalis* this is always the larger. "Die Theilung erfolgt aber nicht ganz gleichzeitig, die grössere Furchungskugel theilt sich vielmehr etwas früher als die kleinere." In *Planocera* this succession is not so marked as in *Discocalis*. In many cases the two spindles are in the same phase at the same time.

The cells resulting from this second cleavage are much more unequal in size than those of the first division. Each of the two cells buds off a smaller cell in a laetropic direction (Pl. XXXV, figs. 5 and 6). As Lang has found in *Discocalis*, the two spindles of this division cross at an angle. Viewed from the side these spindles form an X (fig. 5). It thus happens that the two smaller cells lie at a higher level than the two larger. The former tend to meet in a point at the animal pole (fig. 6). Before the next division they move downwards a short distance, so that a portion of the first furrow is visible between them and forms a short polar furrow. Viewed from the vegetative pole the two larger blastomeres always meet in a line the so-called vegetative polar furrow ("Brechungslinie," Rauber (82), or "Querfurche," Rabl (79)). As in the case of all dextral mollusks and in annelids, this polar furrow turns to the right when viewed in the plane of the first cleavage.

The two smaller blastomeres, *A* and *C*, are approximately equal in size (fig. 6), while of the remaining two, one is significantly larger than the other. The larger of these, *i.e.*, the largest of the four cells, is the one denoted by *D*, and lies on the posterior side of the egg. The next largest cell, *B*, is anterior, while the two smaller cells, *A* and *C*, are lateral in position. It thus happens that the larger of the two blastomeres in the two-cell stage gives rise to the posterior and right blastomeres, *D* and *C*, while the smaller forms the anterior and left blastomeres, *B* and *A*. Thus the first cleavage separates the posterior and right sides from the anterior and left.

THIRD CLEAVAGE—FOUR TO EIGHT CELLS.

The third cleavage is strongly dextrotropic. The resulting cells, 1*a*, 1*b*, 1*c* and 1*d*, come to lie, when completely separated, in the furrows between the macromeres (fig. 8). They retain this position only until after the next cleavage. As has been found in the case of other spirally cleaving eggs, the resulting position of the blastomeres here is not due to surface tension alone. The spindles from the moment of the breaking of the nuclear membranes indicate clearly the direction the cleavage is to take (comp. fig. 12). It is thus a phenomenon inherent in the cell structure, although surface tension no doubt plays a part in giving the blastomeres their final position.

The divisions of the four blastomeres do not usually take place synchronously. Lang gives the following rhythm for *Discocalis*. The largest cell (*D*) divides first, next the anterior large cell (*B*), and after these have divided the lateral cells *A* and *C* divide at about the same time. In *Planocera inquilina* as a general rule the same rhythm holds true, although there are often exceptions to it. The spindles are usually present in all four of the cells at the same time, but those of the two larger cells are nearly always more advanced. The fact that in the great majority of cases this rhythm holds true is exceedingly interesting. Lang (84) was the first to point out that there was such a constant rhythm in the divisions of embryonic cells. Lang found that not only did this succession hold good for divisions of the blastomeres where there is an actual difference in size, but also that the descendants of these cells divided in the same order (see quotation p. 526).

Since that time many other observers have found a more or less similar and constant rhythm in various animals, *e.g.*, Lillie (95) in *Unio*, Jennings (96) in *Asplanchna*, Child (00) in *Arenicola*, and Nelson (04) in *Dinophilus*. In *Unio*, *Arenicola* and *Dinophilus* the order is *D*, *C*, *A*, *B*; in *Asplanchna* it is *D*, *C*, *B*, *A*; while in *Discocalis* and

Planocera it is *D*, *B* (*A*, *C*), the latter two cells dividing at about the same time. It is evident that this is correlated with the larger size of certain blastomeres. It is thus in contradiction to Balfour's (80) law, that a greater amount of yolk retards the rapidity of cleavage. Kofoed (94) has suggested that this difference in rapidity of cleavage is due to the greater absolute amount of protoplasm in the larger cells.

In general this rhythm can be recognized for six or seven cleavages in *Planocera*, although, as stated, there are often exceptions to it.

The cells of the first quartet are only slightly smaller than the cells of the basal quartet (fig. 8). The inequality, while sufficient to be easily recognized, is not so great in this species as in *Discocalis* and most other polyclads. Girard (54) has described the cleavage of *Planocera elliptica* as total and equal. In all others so far as known it is unequal. All the cells of the first quartet are approximately equal in size (fig. 8), notwithstanding the inequality of the cells from which they arose. Of these cells in *Discocalis* Lang says (p. 331): "Anscheinend sind die vier Ur-Ectodermzellen [first quartet] gleich gross; es ist aber sehr leicht möglich, dass sie in Wirklichkeit ähnliche Grössenunterschiede zeigen, wie die vier grossen Blastomeren . . . weil sie bei ihren weiteren Theilungen ganz genau demselben Rhythmus folgen, wie die vier grossen Blastomeren."

FOURTH CLEAVAGE—EIGHT TO SIXTEEN CELLS.

The next cleavage is initiated by the division of the largest of the macromeres, *1D*. This division is followed very closely by the cleavage of *1B* (Pl. XXXVI, fig. 9). Before the daughter cells *2d* and *2b* have been completely separated spindles appear in the two lateral cells *1A* and *1C*. The formation of this second quartet takes place in a laetotropic direction (fig. 9). By the separation of these cells, the cells of the first quartet are pushed towards the left, thus in the opposite direction from which they were given off. While the macromeres are dividing the first quartet begins to divide, also in a laetotropic direction (fig. 9). These cells divide very nearly synchronously, but in very nearly all cases the spindle of the posterior cell *1d* is further advanced than that of the others (fig. 9). The result of this division of the first quartet is eight cells very nearly equal in size (fig. 10). When the divisions are completed the upper or apical cells have rotated almost 45 degrees to the left, so that they now occupy a position very nearly over their respective macromeres (comp. fig. 12). They would no doubt occupy such a position were it not for the inequality of the macromeres, which

becomes more marked with the separation of each quartet. The distal cells of this division, viz., $1a^2$, $1b^2$, $1c^2$ and $1d^2$, correspond in their method and time of origin to the "Trochoblasts" of annelids (Wilson, 92) or the "Turret cells" of mollusks (Conklin, 97). Their future divisions correspond more closely perhaps to the trochoblasts. Since, however, no structure directly homologous with the prototroch is recognizable in the polyclad larvæ, we cannot say that these cells are homologous with those of annelids.

The cells of the second quartet are slightly smaller than the original cells of the first quartet.

FIFTH CLEAVAGE—SIXTEEN TO THIRTY-TWO CELLS.

In the next cleavage each of the sixteen cells divides so that the ideal thirty-two-cell stage is realized. Again the cells of the posterior quadrant *D* divide slightly in advance of the others. The first cell to divide is the large macromere $2D$, which sends off in a dextrotropic direction a small cell $3d$; $3b$ of the anterior quadrant is next separated off. The other two cells of the third quartet may not come off until after the cells of the first and second quartets have divided (fig. 14).

The cells $2d$ and $2b$ begin to divide shortly after the furrows for $3d$ and $3b$ are formed. The divisions of the second quartet are nearly meridional, but, as Pl. XXXVII, figs. 16 and 19 show, the right cell is slightly higher than the left, so that the division is dextrotropic. The lower distal cells, $2a^2-2d^2$, are the larger (fig. 16). Before this division has proceeded far the apical or stem cells of the first quartet divide in a dextrotropic manner (fig. 12). The distal cells, $1a^{1.2}-1d^{1.2}$, are somewhat smaller than the remaining apical cells (fig. 13). The former lie in the angles between the latter, and the cells $1a^2-1d^2$ are pushed out until they lie opposite the ends of the apical cells. By this time the other two cells of the third quartet, $3a$ and $3c$, have usually been constricted off (fig. 14). Next the remaining cells of the first quartet, $1a^2-1d^2$ (trochoblasts), divide in a dextrotropic manner. In this case the lower distal cells, $1a^{2.2}-1d^{2.2}$, are somewhat smaller than their stem cells (fig. 13).

At this stage the cells of the third quartet, $3a-3d$, are the smallest cells present. The formation of this third quartet in polyclad eggs was first observed by Lang, the older workers having overlooked it. Lang calls this quartet the primitive mesoderm of the second order.

The thirty-two cells are distributed as follows:

First quartet,	16 cells.
Second quartet,	8 "
Third quartet,	4 "
Basal quartet,	4 "
	— "
	32 "

From the above it is noticeable that the cells of the first quartet show a tendency towards rapid division. In a number of mollusks, e.g., *Neritina* (Blochman, 81), *Unio* (Lillie, 95), *Crepidula* (Conklin, 97) and *Fiona* (Casteel, 04), the first quartet has divided only once before the third quartet is formed. In *Umbrella* (Heymens, 93) and *Urosalpinx* (Conklin, 91) the first quartet does not divide at all until after the third is formed. On the other hand, in the polyclads so far studied (Lang, 84), in *Neries* (Wilson, 92), *Limax* (Kofoid, 94), and in *Dinophilus* (Nelson, 04), the first quartet divides twice before or at the time the third is forming. As Conklin (97) has pointed out, this indicates the general rate of development of the upper hemisphere. In *Planocera* this is exceedingly rapid as its further history will show.

FORMATION OF THE FOURTH QUARTET—THIRTY-TWO TO FORTY CELLS.

After the thirty-two-cell stage the divisions become more or less irregular; certain cells divide several times before others divide at all. After the completion of the thirty-two-cell stage the next cells to divide are the large basal quartet cells, 3D and 3B. The nuclei of these cells have moved from the upper to the lower edge of cells, and when the spindle forms it reaches from the centre of the cell downward to its lower margin (figs. 15 and 16). These spindles are very nearly radial in position. Concerning them in *Discocalis* Lang says (p. 335): "Nur sehr schwach ist die Abschnürung in der Richtung einer rechts gewundenen Spirale (wenn wir das Ei so orientiren, dass der orale Pol unten, der aborale oben liegt, und der Beobachter in der Achse des Eies steht) angedeutet." By comparing figs. 20 (Pl. XXXVII), 25 and 26 (Pl. XXXVIII) and 30 (Pl. XXXIX), it will be seen that the small macromeres show a twisting towards the left as seen from below, thus showing that the fourth quartet arose by dextrotropic cleavage. As may be seen from the spindles in fig. 15, the turning of the spindle itself is very slight indeed.

As figs. 20, 25, 26 and 30 show, these lower cells are very small compared to the cells from which they arose. Wilson (98) has designated these small cells the macromeres, and the large cells from which they arose the fourth quartet. As will become evident later on, I have

additional evidence which points to this as the true interpretation. Although the name "macromere" is evidently a misnomer in this case, it nevertheless seems well to retain the name for these small cells. Wilson (98, p. 21) calls attention to the fact that these macromeres "are relatively not very much smaller than in some of the mollusks," for example *Planorbis* (Rabl, 80). Judging from the figures, however, the relative size of the cells varies considerably in different species of polyelads. In *Planocera inquilina* these cells are relatively very small as compared to the fourth quartet.

Hallez (79) first interpreted these small cells at the oral pole (macromeres) as mesoderm. Selenka in his earlier work called them pharyngeal cells, and in his later papers primitive endoderm cells. Goette (82) and Lang (84) designate them as lower endoderm, and believe that they give rise to part of the alimentary canal. These four cells form one of the chief landmarks up until a very late stage of segmentation. At a time when the ectoderm is well established in a layer, and just before it begins to invaginate at the lower pole to form the pharynx, one can still make out these four cells. Up until this time they have not divided. Their later history is exceedingly difficult to follow. I am inclined to the view that these cells do not take part in the formation of any structures in the embryo of *Planocera*, but that they degenerate and that their substance is absorbed by the endoderm cells. The reasons for this view are, first, that they cannot be traced to any organ, and, secondly, that the nuclei of these cells from the time of their formation until the last trace that can be found of them show increasing evidence of degeneration. Soon after these cells are formed it can be seen that the chromatin is massed on one side of the nucleus in a dark staining mass (figs. 17 and 20, Pl. XXXVII, and fig. 25, Pl. XXXVII, etc.). It is this marked evidence of degenerating nuclei that enables one to follow these cells even until the ectoderm has reached this pole of the egg. In the other nuclei of the egg the chromatin is more or less evenly distributed in granules often along distinct linear threads. We thus have the remarkable and unique phenomena of the "macromeres" degenerating without giving rise to any part of the embryo. Evidently the function of the macromeres in this case has been taken over by the cells of the fourth quartet. These latter cells, as the figures show, are by far the largest cells in the embryo and contain the great bulk of the food yolk. In fact three of these cells, 4a, 4b and 4c, probably function entirely as the bearers of yolk, and if they do take part in the formation of the alimentary canal it is only a minor part. The history of the other member of the fourth quartet, 4d, is of especial interest and will

be dealt with later. From the fact that this cell gives rise to both endoderm and mesoderm we may call it the "Mesentoblast" (Conklin, 97).

HISTORY OF THE FIRST QUARTET—APICAL CELLS.

After the first two macromeres, 4B and 4D, are formed and before the other two cells, 3A and 3C, have divided, spindles appear in the four stem cells of the first quartet, $1a^{1.1}-1d^{1.1}$. The division of these cells takes place in a very marked læotropic direction. The results of this division are four very small cells, $1a^{1.1.1}-1d^{1.1.1}$, lying at the proximal end of the spindle or just over the animal pole, and four much larger distal cells, $1a^{1.1.2}-1d^{1.1.2}$. As fig. 18, Pl. XXXVII, shows, the læotropic direction of the spindle is so marked that the cell $1a^{1.1.1}$ comes to lie in front of $1d^{1.1.2}$ and $1b^{1.1.1}$ in front of $1a^{1.1.2}$ and so on, so that there is a rotation of 45 degrees.

These small cells at the animal pole have been observed by Selenka and Lang in polyclads, and designated by Selenka as the apical or crown cells (Scheitelzellen). Selenka described these cells as arising from the stem cells of the first quartet, *i.e.*, $1a^{1.1}-1d^{1.1}$, as I have done. Lang, on the other hand, says that they arise from the cells which alternate with these stem cells, *i.e.*, ae_3 , be_3 , ce_3 and de_3 of his system, or $1a^{1.2}$, $1b^{1.2}$, $1c^{1.2}$ and $1d^{1.2}$ of our system. He states that these cells $1a^{1.2}-1d^{1.2}$ send in processes between the stem cells, and from the ends of these processes the small apical cells arise. Lang describes this process in considerable detail, and while it is possible he is right for *Discocalis tigrina*, I think it is very unlikely.

Lang's observations were all made on the living eggs and not checked with stained material. I first observed these divisions in the living egg of *Planocera* and came to the same conclusion as Lang, *viz.*, that the apical cells came from the cells $1a^{1.2}-1d^{1.2}$. It was only later when I studied the preserved material of this stage that it became unmistakably evident that it was the stem cells $1a^{1.1}-1d^{1.1}$ that were dividing (fig. 18). The appearance in the living egg is very deceptive owing to the great angle through which the spindles turn and to the small size of the resulting apical cells.

These apical cells consist of a well-formed, normal-sized nucleus and a very small amount of cytoplasmic material. In the stained eggs it is only seldom that one can see anything at all of the cell boundaries. Consequently in most of the drawings only the nuclei of these cells are represented.

In their method and time of origin and in their relative size these

cells correspond closely with the "apical rosette" of annelids. In *Dinophilus* (Nelson, 04) these cells arise by exactly the same division as in *Planocera*. In *Nereis* (Wilson, 92) they arise one division earlier, *i.e.*, by the division of $1a^1-1d^1$. In many mollusks, cells of this same lineage $1a^{1.1.1}-1d^{1.1.1}$ are not formed until much later in the development, *e.g.*, in *Crepidula* at the eighty-eight-cell stage, in *Fiona* at the sixty-four-cell stage, etc. The formation of these small apical cells in the polyclad embryo is an interesting and significant phenomenon, marking in another detail the close resemblance between the early cleavage of these platodes and that of annelids and mollusks. The fate of the apical rosette in the majority of annelids is the formation of an apical sense organ with an apical tuft of cilia. In *Capitella* (Eisig, 98) and *Dinophilus* (Nelson, 04) the apical plate does not bear a tuft of cilia. Conklin (97) finds that these cells form an apical sense plate in the *Crepidula veliger*, without, however, bearing a bunch of large cilia.

As to the ultimate fate of these cells in *Planocera*, I must again disagree with the previous students of polyclad embryology. Both Selenka (81) and Lang (84) state that these cells sink down and are finally covered over by the other ectoderm cells. Thus Lang (p. 337) says: "Ihre Abschnürung geschieht nicht ganz gegen den aboralen Pol zu, sondern etwas nach innen, gegen die Furchungshöhle, so dass sie, wie auch Selenka bemerkt, den Boden einer napfartigen Vertiefung am aboralen Pol bilden. . . . Da später in der Nähe der Stelle, an der sich die Scheitelzellen gebildet haben, in besonderen Zellen des Ectoderms die Augen entstehen, so wäre es möglich, dass aus ihnen Theile des Nervensystems, vielleicht der sensorielle Theile des Gehirns (oberes Schlundganglion?) entstünden." Again, I believe the deceptive conditions of the living egg have led both Selenka and Lang astray. It is true that by reason of the small size of these apical cells the surrounding cells somewhat overtower them and form a "bowl-like depression" (Pl. XXXVII, fig. 21). However I do not find, as Lang states, that by the further division of the first quartet cells the apical cells are covered over and so sink beneath the surface of the ectoderm. In focusing on an egg from the animal pole in stages shown in Pl. XXXVIII, figs. 22, 23 and 24, the first nuclei to come into view are those of the apical cells. Furthermore these cells tend to move out from the animal pole and remain on the surface (figs. 24, 29, 31). In the later stages of segmentation they cannot be definitely distinguished from other cells of the same size which come to lie near them. The further division of the ectoderm cells in this region do not show any

indications of covering over these cells. I believe that these apical cells form a part of the ectodermal covering of this region of the embryo. And furthermore I believe that these are the first of a series of divisions occurring from now on in all the ectodermal cells, by which a small epithelial cell is cut off towards the exterior and a larger cell remains somewhat deeper down. It is by this kind of divisions that nearly all of the later ectodermal layer is formed. These divisions will be discussed farther on in this paper.

Whether these apical cells form a definite sense organ or not I am unable to say. It is in this region, as Lang points out, that the eyes arise and that later the tentacles of the adult appear. However, the exact fate of these cells is mere speculation, since I have found it impossible to distinguish them in the late segmentation.

FURTHER HISTORY OF FIRST QUARTET.

We have so far followed the divisions of the first quartet until it is composed of twenty cells, and to the time when there are forty cells in the embryo. The last cells to be formed were the small apical cells, $1a^{1.1.1}-1d^{1.1.1}$. Very soon after this the cells $1a^{1.2}-1d^{1.2}$ divide in a dextrotropic manner into two almost equal cells. The lower cells, $1a^{1.2.2}-1d^{1.2.2}$, appear slightly smaller than the upper cells (figs. 21, 22). Next, and sometimes coincident with the last division, spindles appear in the large cells $1a^{1.1.2}-1d^{1.1.2}$. These divide in a dextrotropic direction into two very unequal cells (figs. 22, 23). The upper cells, $1a^{1.1.2.1}-1d^{1.1.2.1}$, are much the smaller and lie on the surface of the egg between the cells $1a^{1.1.2.2}-1d^{1.1.2.2}$ (fig. 24). While this division is occurring the cells $1a^{2.1}-1d^{2.1}$ are dividing in a læotropic direction (figs. 22, 23). Shortly after these divisions are completed the cells $1a^{2.2}-1d^{2.2}$ divide dextrotropically (fig. 24). In this case the lower cells, $1a^{2.2.2}-1d^{2.2.2}$, form very small cells which lie on the surface of the egg (figs. 24, 27).

At this time there are thirty-six cells in the first quartet and about sixty-six cells in the entire embryo. This fact indicates clearly the very rapid development of the upper hemisphere. In *Crepidula* at the sixty-eight-cell stage there are only sixteen cells in the first quartet. In *Fiona* at a similar stage there are twenty cells in this quartet. In *Nereis* at the fifty-eight-cell stage there are thirty-two cells in the first quartet, and a similar number in *Dinophilus* at the sixty-five-cell stage.

Soon after the last division the cells $1a^{1.1.2.2}-1d^{1.1.2.2}$ divide again in a slightly dextrotropic direction (fig. 28). This time the distal cells, $1a^{1.1.2.2.2}-1d^{1.1.2.2.2}$, are the smaller and lie opposite the cells from which

they have been derived (Pl. XXXIX, figs. 29, 31). At this time there are three circles of four small cells each, lying on the surface of the egg and arranged around the animal pole (figs. 29, 31). These twelve cells have been derived by three successive divisions of the large stem cells of the first quartet, $1a^{1.1}-1d^{1.1}$. The inner circle consists of the small apical cells already described. The middle circle consists of the cells $1a^{1.1.2.1}-1d^{1.1.2.1}$, which were derived next and are the largest of these twelve cells. The outer circle was derived at the last division described. At the next division of these stem cells four more small cells are cut off to the exterior.

At the same time that the last division described is occurring, the cells $1a^{1.2.1}-1d^{1.2.1}$ divide in a laetropic direction (fig. 28) into nearly equal moieties. With the completion of the above divisions there are forty-four cells in the first quartet, eleven in each quadrant. After a short resting period the cells $1a^{1.1.2.2.1}-1d^{1.1.2.2.1}$, which have been so actively dividing, prepare to divide again. This time they bud into the interior of the egg four comparatively large cells, $1a^{1.1.2.2.1.2}-1d^{1.1.2.2.1.2}$, the primitive ganglion cells. The outer smaller cells of this division, $1a^{1.1.2.2.1.1}-1d^{1.1.2.2.1.1}$, form the fourth circle of small cells about the animal pole. This is as far as I have been able to follow accurately the divisions of these cells, and it is possible that the cells here designated primitive ganglion cells may still bud one or two generations of ectoderm cells to the exterior. The four cells which are to form the ganglion divide repeatedly. Their individual divisions have not been traced, but four strings of cells can be distinguished for some time, each of which is the result of the subdivision of one of these primitive ganglion cells. These ganglion cells lie at first just above the mesoderm cells, $4d^{2.1.1}$ and $4d^{2.2.1}$, and it is extremely difficult to differentiate the later divisions of these cells.

In the divisions of this first quartet we have had a number of examples of the process already alluded to, in which a very small cell is budded to the exterior of the egg and partially covers the larger, deeper lying moiety. Other examples of this same phenomenon will be described in the history of the second quartet. The four sets of four small cells each which, beginning with the apical cells, are budded off in rapid succession from the large stem cells of the first quartet, $1a^{1.1}-1d^{1.1}$, are among the more striking examples of this phenomenon. These sixteen cells (and possibly more) very nearly cover the aboral surface of the egg, and by their further divisions the ectoderm of this region is formed. Other cells of this first quartet show the same process more or less strikingly. For instance, by the divisions of $1a^{2.2}-1d^{2.2}$ a very

small cell is cut off at the lower edge (figs. 24, 27). The same is true of the divisions of $1a^{2.1}-1d^{2.1}$. On the other hand the cells $1a^{1.2}-1d^{1.2}$ do not show such an unequal division. These latter cells have been traced accurately through only three or four divisions, but the resulting cells are all more or less equal in size and apparently will enter directly into the formation of the ectoderm. This latter process, *i.e.*, the equal division of the ectoderm cells, is much more in accord with what has been found in annelids and mollusks than is the former.

HISTORY OF THE SECOND AND THIRD QUARTETS.

Just after the formation of the apical cells, and at about the time the large cells 3A and 3C are dividing, the larger cells of the second quartet, $2a^2-2d^2$, divide in a nearly vertical direction (fig. 17). In many cases the spindles show a slight dextrotropic turn. Since the resulting distal cells $2a^{2.2}-2d^{2.2}$ come to lie in the furrows between the large cells of the fourth quartet, they are somewhat shifted in position to accommodate themselves to the inequalities of these large cells.

It thus happens that after the cells are separated some may be to the right of the upper cell and others to their left. The division is always nearly radial, but is still to be considered as belonging to the series of spiral divisions.

With this we have traced the divisions of the second quartet until there are twelve cells present, three in each quadrant and all on the surface of the egg.

Just after the mesentoblast cell has divided for the first time the cells $2a^1-2d^1$ divide almost vertically but in a slightly laetotropic direction. The lower or distal cells, $2a^{1.2}-2d^{1.2}$, are very much smaller than the upper ones (fig. 21). The former lie on the surface of the latter. This is another example of the cutting off of a small cell to the exterior. At about this time, sometimes before and sometimes afterwards, the small cells $2a^{2.2}-2d^{2.2}$, lying in the furrows between the four primary cells of the fourth quartet, divide. The direction of the nuclear spindle is nearly vertical (figs. 26, 27). The two cells seem nearly equal in size. The lower cells, $2a^{2.2.2}-2d^{2.2.2}$, reach almost to the upper edges of the macromeres (figs. 26, 30). The descendants of these cells are the first to reach the lower pole of the egg, and form without a doubt a considerable portion of the ectodermal pharynx.

Shortly after these two divisions the large cells of the second quartet, $2a^{2.1}-2d^{2.1}$, divide in a dextrotropic manner, the two cells varying only slightly in size (figs. 25, 27). At this time there are twenty-four cells in the second quartet, six in each quadrant, and about seventy-eight

cells in the entire embryo. So far all the cells of this quartet lie on the surface of the egg. At the next division in this quartet however the cells $2a^{1.1}$ – $2d^{1.1}$ cut off a small cell to the exterior and the major portion of the larger cell pushes inwards (figs. 30, 32), so that they become almost covered by the surrounding cells. Unfortunately I have not been able to follow the further divisions of these cells with certainty. At a later stage one finds one or two more small cells lying over these larger, deeper lying ones. For this reason I am led to suspect that these cells bud off one or two more ectoderm cells. The major portions of these four cells remain on the interior of the egg and form a portion of the mesoderm. Since these cells when first budded in are well towards the lower (oral) side of the egg, it is very probable that they form a portion of the mesoderm around the blastopore. In later stages (Pl. XL, figs. 36–39) a considerable amount of mesoderm is found in this region. This later supplies the muscles and other mesodermal structures connected with the pharynx. The other mesoderm cells found just beneath the ectoderm in these stages are derived from the divisions of $4d$, as will be described shortly.

This account of the second quartet agrees closely in its main features with that of Wilson (98) for *Leptoplana*. Wilson, however, believed that these second quartet cells, on the interior, multiplied rapidly and formed the entire mesoderm of the body. The development of the remainder of the mesoderm will be dealt with farther on, and it need only be pointed out here that this account confirms Wilson's with regard to ectoderm arising from the second quartet. In contradiction to Lang (84), who believes that the whole of the second and third quartets formed mesoderm, we find here only a relatively small portion of the second quartet budding to the interior. By far the greater bulk of this quartet is ectodermal.

With regard to the third quartet, this is in all probability entirely ectodermal. The cells of this quartet when first formed are relatively very small. These cells divide in a nearly radial direction (slightly dextrotropic) at about the seventy-four-cell stage (fig. 26). Further divisions of this quartet have not been traced accurately. At later stages, however, one or both of these cells in each quadrant have divided and all their progeny remain on the surface. There is no indication that any of these cells pass to the interior. Their small size and epithelial character indicate that they are purely ectodermal.

HISTORY OF THE FOURTH QUARTET—THE MESOBLAST.

Shortly after the apical cells are formed and the cells $2a^2$ – $2d^2$ have

divides each time a stage with forty-four cells, the mesenchymal cell 44 divides, with the nuclear spindle lying in a vertical direction. In this division a single large cell is budded into the interior of the embryo and occupies most of the previous segmentation cavity (text fig. 3 and Pl. XXXVII fig. 16). The spindle for this division is

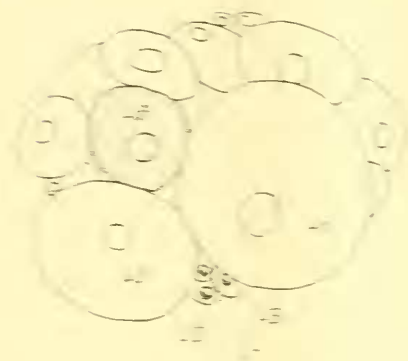


FIG. 3.—Global section of embryo viewed from the right side. Shows the mesenchymal cell 44 just after it has been budded into the segmentation cavity (cf. fig. 16, Pl. III).

oriented apically and lies exactly in the latter's median longitudinal plane of the embryo. So far as one can observe it is inclined neither to the right nor the left, and the resulting cell 45 seems to sit directly over 44 (figs. 24, 25). This is thus the first division which shows a strict deviation from the spiral type. The cell 45 on the interior of the egg is about three-fourths the size of the outer cell 48. Very soon after its formation 45 divides by a horizontal division into two approximately equal cells (figs. 24, 25 and 26). This division is strictly bilateral, the future median plane of the embryo passing between these two cells (figs. 25 and 26). Just as this division is completed the cell 48 divides parallel to the last (fig. 26). The two cells resulting from this division are also equal in size (fig. 26).

Of these three divisions of 45 only one, the last, has been previously observed. Haller, Seeman, Goette, Long and Wilson have all found that this posterior cell divides in a horizontal direction into two more or less equal portions. This peculiar division of 45 is similar to that of a somite and notochord cell Meck (45) to produce cells to give rise to the somites. No one, however, has hitherto shown that it budded into the interior of the egg.

Following these stages the divisions of 45 bud there are four cells.

Two of these $4b^{1-2}$ and $4b^{3-4}$ are the larger and lie on the surface of the egg. These two cells are purely ectodermic and hence we may leave their future history for the present. The other two cells $4b^{5-6}$ and $4b^{7-8}$ lie entirely concealed (figs. 29-31) and are still probably mesenchymal. Soon after these cells are formed they again divide. This time broad spindles point towards the middle of the embryo and the division is very unequal (fig. 31). Two very small cells $4b^{9-10}$ and $4b^{11-12}$ are thus budded into the interior of the embryo and lie very near its centre. The nuclei of these cells are very small and stain very intensely. Their position is constant, always lying just posterior to the very large nucleus of $4a$, which has been crowded towards the centre of the egg, since these small cells do not divide again for a very long time they form an excellent landmark in the later stages. In fact these two minute cells lying near the centre of the embryo can be followed until just before the pharyngeal invagination. Fig. 36a shows two small cells lying just above the large mass of endoderm cells. From their position and character I believe these are the cells $4b^{9-10}$ and $4b^{11-12}$. At this time, which is immediately preceding the ectodermal invagination from the oral pole, the mesoderm cells have migrated to the sides of the embryo and lie just beneath the ectoderm. The whole centre of the egg is occupied by the homogeneous yolk spheres in which the somewhat shrunken nucleus of $4a$ can be seen. Lying just below this nucleus are these two small cells which I believe take part in the formation of the alimentary canal. From their first appearance, the character of their nuclei (small, dense and intensely staining) indicates that their fate may be different from the other descendants of $4b$. They are formed and remain in exactly the path later traversed by the alimentary canal while the other descendants of $4b$ move towards the periphery of the egg.

The much larger cells $4b^{1-2}$ and $4b^{3-4}$ from which these small endoderm cells arise, are now entirely mesenchymal. After a short resting period they again divide, giving rise to two rather small cells $4b^{1-2-3-4}$ and $4b^{5-6-7-8}$ lying above and somewhat posterior to the small ectoblasts $4b^{9-10}$ and $4b^{11-12}$ (fig. 34). The nuclei of these small mesoblasts are larger and more rounded in appearance than those of the previously described ectoblasts ($4b^{9-10}$ and $4b^{11-12}$). Not long after this the larger cells $4b^{1-2-3-4}$ and $4b^{5-6-7-8}$ again divide. The spindles for this division are shown in optical section in fig. 34. Pl. XII, fig. 35, represents an optical section of an egg somewhat older than the preceding one. In this figure we are looking down nearly upon the vegetative pole of the egg. The position of the large shrunken nuclei of $4a$, $4b$

and 4c are shown in dotted outline. Just posterior to the nucleus of 4b lie the two small cells $4d^{2.1.1}$ and $4d^{2.2.1}$ with the darker staining nuclei. Just posterior to these cells are the slightly larger cells $4d^{2.1.2.1}$ and $4d^{2.2.2.1}$ mentioned above. Finally, extending dorsally and anteriorly from each of these are two cells which represent the results of the division just mentioned and for which the spindles are shown in figure 34. The resemblance to the so-called "mesoblast bands" of annelids and mollusks is, I think, evident. In many cases, however, the "band" formation is not so marked as in this egg, which was chosen for drawing on account of its regularity. In many cases the meso-

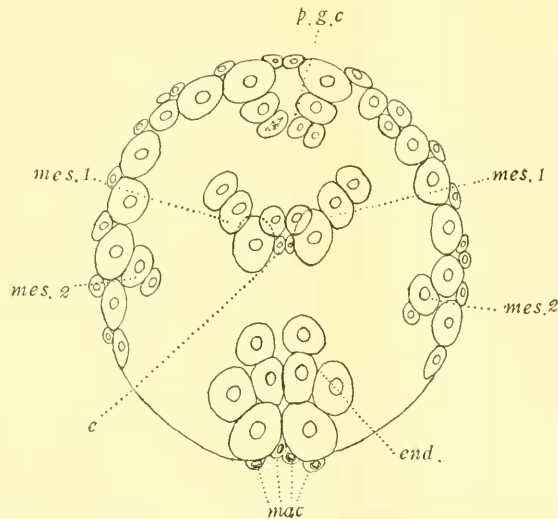


Fig. 2.—Schematic optical section of an egg viewed from the posterior side. To show the relation of the ectoderm, mesoderm and endoderm. *Mes. 1*, Mesoderm derived from $4d^2$. *Mes. 2*, Mesoderm derived from second quartet cells. *End.*, Endoderm from $4d^1$. *e*, Entoblasts from $4d^2$. *p.g.c.*, Primitive ganglion cells.

blast cells do not form such evident bands. Instead the nuclear spindles in the cells $4d^{2.1.2.2}$ and $4d^{2.2.2.2}$ lie at varying angles. This is shown by the direction of the spindles in these cells in fig. 34. The result tends to be a cluster of cells rather than symmetrical bands. The lineage of these cells becomes too involved to trace further with any accuracy. They lie well towards the dorsal side of the egg, and become more or less confused with the cells which are now budding in from the first quartet to form the ganglion. Nevertheless a fairly definite group of cells can be found in this region during several of the succeeding stages. I have attempted to represent something of the

relation of these cells in the diagrammatic optical section (text fig. 2). Text fig. 3 represents an actual optical section of a somewhat later stage. Here the large nuclei of $4a$, $4b$, and $4c$ are again shown, while lying just above them is a group of mesoderm cells derived from the division of $4d^2$. The mesoderm now consist of a large number of cells. From this time on these begin to spread out and gradually form a layer of cells lying just beneath the ectoderm.

While I have not been able to follow the exact lineage of these cells, I think there is no doubt that the greater portion of the mesoderm of the body arises from the posterior mesoblasts, while as stated before only the mesodermal structures in the region of the pharynx arise from cells of the second quartet.

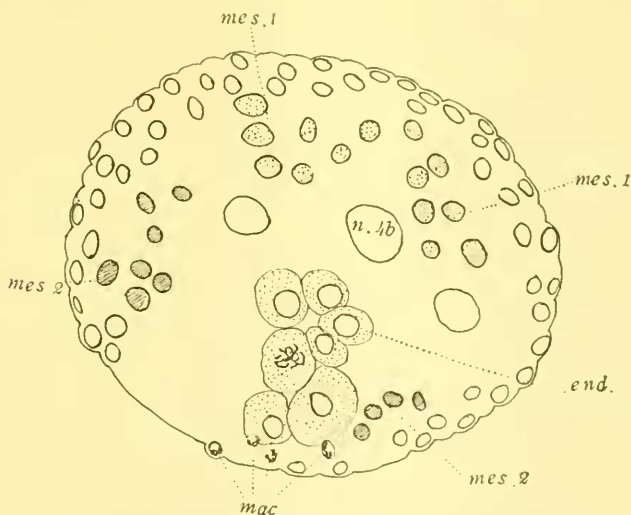


Fig. 3. —Optical section of an egg viewed from the posterior side. *end.* Group of endoderm cells derived from $4d^1$. Below these are the degenerating nuclei of the four macromeres. Near the middle of the egg are the three large nuclei of $4a$, $4b$ and $4c$. Above these are shown the nuclei (stippled) of a few of the mesoderm cells derived from $4d^2$, *mes. 1*. Toward the vegetative pole are mesoderm cells derived from the second quartet, *mes. 2*.

Text fig. 3 shows a number of cells lying further towards the vegetative pole than the derivatives of $4d^2$ have yet reached. These cells undoubtedly arise from the further proliferation of the second quartet cells, $2a^{1.1.2}$ – $2d^{1.1.2}$, which, as we have shown (p. 535), were budded into the interior of the embryo (cf. fig. 32). These second quartet cells, $2a^{1.1.2}$ – $2d^{1.1.2}$, when first formed lie considerably below the equator of the egg. With the overgrowth of the ectoderm they are carried

further down to the region of the blastopore. Thus the large group of mesoderm cells found later around the pharynx arise, in large part at least, from the second quartet cells. It is, however, quite possible that some of the derivatives of $4d^2$ later wander into this region also.

We have now followed the history of the mesoderm from its origin until it forms a layer of cells about the embryo just beneath the ectoderm. We may leave the discussion and comparison with other forms until after we have traced the history of the endoderm.

THE ENTOBLAST.

The alimentary canal of the polyclads has been derived in various ways by different investigators. Vaillant (68) and Keferstein (68) observed that at the time the embryo began to rotate within its membranes there was a mass of roundish fat-like spheres on the interior. Hallez (78 and 79) observed drops of an egg-white-like substance on the inside of the embryo, surrounded by a one-cell layer of the alimentary canal. The cellular elements of the canal he derived rather doubtfully from the four small cells at the oral pole (macromeres). Selenka (81c) came to a similar conclusion, *i.e.*, that the entire canal arose from the four small "primitive endoderm cells" (macromeres). These were carried into the embryo by the pharyngeal invagination and rapidly spread over the large yolk cells (fourth quartet). According to this investigator these yolk cells break up without nuclear division into a large number of yolk spherules and serve as food for the developing endoderm. They give rise to no morphological structure of the embryo. Selenka (81) says that as soon as "die Nahrungsdotterzellen in ein Dutzend oder mehr ungleich grosse kernlose Kugeln zerfallen sind, beginnen die vier Ur-Entodermzellen (macromeres) ihre Theilung und Wanderung. Zunächst strecken sie sich in die Länge, entsenden Ausläufer und breiten sich auf den benachbarten Dotterkugeln aus."

Goette (82) in *Stylochus pillidium* finds, as already mentioned, upper endoderm cells. From these and from the middle endoderm cells (fourth quartet) small cells are separated, which at first contain some of the fat-like yolk. This soon disappears in these cells and they come to form a definite layer of endoderm. The small lower endoderm cells (macromeres) also take part in forming this layer. Large drops of the homogeneous yolk substance separate from the large middle endoderm cells and are gradually absorbed by the cells of the canal.

Lang (84) in *Discocalis*, it will be remembered, also finds an upper endoderm as well as a lower and a middle layer. Like Selenka, he no longer finds any nucleus in the large cells (fourth quartet) after the

upper and lower endoderm have separated off. With regard to the middle endoderm cells he says, p. 356: "Die in ihnen enthaltenen groben Dotterkörner scheinen miteinander zu verschmelzen so dass die in Frage stehenden Zellen das Aussehen von beinahe homogenen, stark lichtbrechender Fettkugeln bekamen. Ich habe in diesen Kugeln bei ihrem zerfall nie Amphiasier sich bilden sehen, obschon ich aufmerksam danach gesucht habe."

The breaking up of these yolk cells is very irregular. The wall of the alimentary canal, according to Lang, is formed by the cells of the upper and lower endoderm. These increase by division and extend over the yolk spheres. Finally they unite to form a definite layer in which the cell boundaries cannot be distinguished. The endoderm cells have a more or less amœboid character and send out protoplasmic processes over the numerous yolk spheres. According to him the middle endoderm (fourth quartet) contains only yolk granules and does not take part directly in the formation of any organ.

Throughout all these accounts one or two phenomena are constant. *e.g.*, the yolk breaks up into a large number of spherules which are later absorbed by the endoderm cells. With regard to the development of the canal itself there is some variation. In all the accounts at least a portion of the canal is derived from the lower endoderm (macromeres). In some cases (*Stylochus* and *Discocalis*) upper endoderm cells are formed from the large yolk cells.

The account which I have to offer of the development of the alimentary canal in *Planocera inquilina* differs from any of the above in several particulars. At the time when the mesoblasts, $4d^{2.1.2}$ and $4d^{2.2.2}$, are preparing to divide, the two large entoblasts, $4d^{1.1}$ and $4d^{1.2}$, are dividing (fig. 33). By this division two large cells, $4d^{1.1.2}$ and $4d^{1.2.2}$, are budded into the interior of the embryo. Soon after this these cells divide again. At a considerably later stage the two cells $4d^{1.1.1}$ and $4d^{1.2.1}$, which remained on the surface after the last division, divide again, budding two more large cells into the lower part of the embryo. At this time or shortly afterwards the ectoderm has covered this region, and all six cells and their descendants originally derived from the two primitive entoblasts, $4d^{1.1}$ and $4d^{1.2}$, are on the interior of the egg.

Text fig. 3 shows in optical section an egg of a considerably later stage, in which a number of cells are lying just above the vegetative pole. These cells, of which there are several more in the egg, all came from the primitive entoblasts, $4d^{1.1}$ and $4d^{1.2}$. By examining a large number of eggs it is found that these cells are in very active division

and soon a large group of cells is found in this region (cf. fig. 36). A careful study of the eggs themselves leaves no doubt but that they all arise from the primitive entoblasts. Pl. XXXIX, fig. 33, shows the spindles for the first division of these cells. I have repeatedly found eggs showing the second and later divisions of these cells, but in every case it has been found impossible to make an intelligible drawing of the egg. The fact that these eggs cannot be rotated under the cover glass has added greatly to the labor of finding eggs suitable for study or drawing. In this case it has effectually blocked all attempts to portray a certain stage. The material itself, however, leaves no doubt that the divisions take place as described. I have attempted to embody the essential facts of these divisions in the schematic text fig. 2.

During all this time the very large nuclei of the three anterior cells of the fourth quartet, 4*a*, 4*b* and 4*c*, can still be found (fig. 34). Previous to this time there has been some shifting of the relative positions of the fourth quartet cells. At the time the mesentoblast cell is budded into the interior (fig. 19) all the four cells of this quartet lie in nearly the same plane. The two lateral cells, 4*a* and 4*c*, are perhaps slightly higher than the others. When 4*d'* divides bilaterally these two cells overlap 4*a* and 4*c*. With the further development of the mesentoblast on the interior of the egg and the ectoblast on the outside, the large cell 4*b* is pressed upwards. A narrow process from this cell runs along the centre of the egg, and in this, reaching almost from one side of the process to the other, lies the enormous nucleus of 4*b* (figs. 32 and 34). The nuclei of 4*a* and 4*c* are also pressed up along the sides, but not so high as that of 4*b*. These three cells are closely crowded together, and while their boundaries remain distinct for some time (fig. 32) they tend to become obliterated.

In the meantime an interesting process has been going on within these cells. As has been noted by all previous students, the yolk granules tend to fuse together, thus forming homogeneous fat-like drops. As Selenka and Lang have noted, this breaking up of the yolk is not accompanied by nuclear division. The process is more or less irregular, but one or two regular features can usually be recognized. The first one of these spherules to be formed is from the anterior and ventral portion of the cell 4*b*. At first the centre of these spheres is composed of granules, while around its periphery the granules dissolve into a fluid substance. Soon afterwards smaller spheres appear in this cell and in 4*a* and 4*c*. No nuclear division is concerned in this process, for the undivided large nuclei of all three of these cells can be followed

to a much later period. In or near the centre of many of these yolk spheres a diffusely staining substance can be found when the egg is well stained and cleared. This is not a nucleus, for it has no regular boundaries and often several such bodies are seen in a single yolk spherule. They do not stain deeply, but appear as a sort of cloudy material. These bodies may be merely the small portions of cytoplasm remaining in these large cells or, what seems more likely to me, they may be of the nature of nuclear sap. If they were of a cytoplasmic nature there is no reason why they should not show in earlier stages. Instead they became evident only after the yolk begins to break up into spherules. In many respects they resemble the archiplasmic material often found around a nucleus just after the nuclear mem-

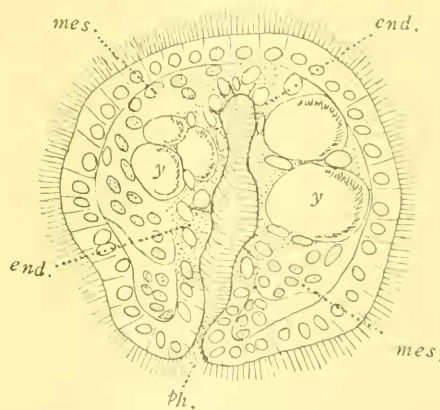


Fig. 4.—Transverse section of an embryo, showing the ciliated lumen of the alimentary canal. The endoderm cells are spreading over the yolk spheres. *end.*, endoderm; *mes.*, mesoderm; *ph.*, pharynx; *y.*, yolk.

brane has broken. Further, the large nuclei, which are at first spherical, in the late stages become irregular in shape or even very much shrunk (fig. 35). That these yolk granules should be broken up through the action of enzymes coming from the nucleus is not at variance with the modern views of nuclear activity.

In such a case it seems probable that this material would become aggregated at those places where the most rapid dissolving action is going on. This offers an explanation for the relatively enormous size of the nuclei of these three cells. These nuclei do not show evidences of degeneration; instead the chromatin granules can be seen scattered through the nucleus, often along distinct linin threads.

After the ectoderm has covered the lower hemisphere of the egg it

begins to invaginate in the region of the four small macromeres. The ectoderm pushes in and forms a small tube, which later becomes the pharynx. Previous to this invagination the endoderm cells derived from the divisions of $4d^{1.1}$ and $4d^{1.2}$ have formed a solid mass of cells in the lower part of the embryo (fig. 36). Soon after the invagination starts, the beginning of a lumen in the endoderm cells is apparent by the separation of the cells (fig. 37). This lumen rapidly becomes large and ciliated throughout (Pl. XL, figs. 38, 39, text fig. 4). The canal is at first bent towards the posterior side of the larva (fig. 38), but with its further development and enlargement it pushes forward under the ganglion (fig. 39). Distinct cell boundaries can seldom be made out in the endodermal portions of the canal. The inner borders of these cells surround the large yolk spherules. In many cases amœboid cells can be seen spread out on the surface of these yolk spheres. In the oldest larvæ which I was able to obtain (fig. 39) the canal showed no indications of the lateral branches which become evident in the adult worm. In these larvæ some of the reduced yolk spheres are still present.

From the account I have given it will be seen that practically all of the alimentary canal is derived from the two primary entoblasts, $4d^{1.1}$ and $4d^{1.2}$. Certainly the larger portion of the canal has such an origin. There are three other possible sources of a portion of the canal, although no one of these forms any considerable amount of it. One is that the pair of small cells, $4d^{2.1.1}$ and $4d^{2.2.1}$, derived from the further division of the mesentoblast, may form a small portion of the endoderm. The chief reason for suspecting this, is that these cells are formed and remain exactly in the path of the future canal. The second possibility is that the three anterior cells of the fourth quartet, $4a$, $4b$, and $4d$, may contribute a small amount to the canal at a late period. I believe, however, that such is not the case. The shrunken nuclei of these three cells, which can be seen at the time the canal is forming (fig. 36), indicate that these cells degenerate without dividing further. That the shrunken condition of these nuclei is not a preparation for karyokinetic division is, I believe, fully established by the fact that these nuclei remain in this shrunken condition for a very long time. Their ability to take up the stain gradually becomes less and less, and the last that can be seen of them (fig. 36) shows a faint, very irregularly outlined nucleus, not at all resembling one about to divide by mitosis.

The third possibility in this connection is that the four small macromeres may, instead of degenerating, contribute a portion to the endoderm. The degenerating character of the nuclei of these cells and

the very small amount of cytoplasm seems to me to preclude such a fate in *Planocera*. I cannot find or at least cannot recognize these cells after the ectoderm has overgrown the lower pole of the egg, and so I am unable to follow their later history. Text fig. 3 gives an accurate representation of the condition of the nuclei of these cells at that stage, which is about as late as I am able to follow them. These nuclei have shown this same condensed condition of the chromatin almost from the time of their formation (cf. figs. 17, 19, 20, 25, 26, 27). These cells are probably carried in with the invaginating pharynx and then absorbed by the endoderm. I have sometimes found what I thought were remains of these cells, but of this I cannot be certain.

DISCUSSION.

We may now return to a comparison of the observations of previous students with regard to the fourth quartet, and especially 4*d*, in other polyclad eggs. As has been already noted (p. 536) the peculiar bilateral division of the posterior cell, 4*d*, has been known since the work of Hallez (79). Hallez believed that this posterior cell divided without an amphiaster, and regarded the product as not equivalent to the other four cells, but in the nature of cell sap. Goette (82) states that in some cases the cells 4*a* and 4*c* of our nomenclature also divide, so that there are seven cells, on the surface of the embryo, formed from this quartet. This happened only in some of the eggs of *Stylochus* (*Stylochopsis*) *pillidium*. In other eggs of the same species only the posterior cell divided. In such cases Goette found that this cell often divided twice, forming four cells, all on the surface of the egg.

Lang (84) finds that in *Discocalis* all the cells of this fourth quartet divide at about the same time, but in different directions. The posterior cell divides horizontally as described, while each of the other three cells buds into the interior of the egg a cell which he calls upper endoderm. Lang says (p. 337): "Es treten in ihnen [fourth quartet of our system] Richtungsspindeln auf, und zwar wieder in der oft angeführten Reihenfolge. Die Richtungsspindel der grössten Stammzelle des Entoderms [4*a*] verlängert sich excentrisch in der peripherischen Verlängerung der Ebene, welche man sich durch diese Stammzelle und die Hauptachse des Eies gelegt denken kann, und welche der Medianebene entspricht." . . . (P. 338): "Unmittelbar bevor sich die grösste Entodermstammzelle [4*d*] in ihre zwei seitlichen Hälften getheilt hat, zeigen sich auch in den drei Uebrigen, Richtungsspindeln, die aber eine ganz andere Direction haben. Sie liegen nämlich parallel zur Hauptachse, d. h. sie zeigen eine dorsoventrale Richtung. Die drei

erwähnten Stammzellen ziehen sich in der That gegen den aboralen Pol zu aus, und schnüren schliesslich je eine kleine Zelle ab, welche unter die Mesodermzellen[second and third quartets] zu liegen kommt." These three cells Lang calls the upper endoderm, while the five cells below he designates as the "mittler Entoderm." Goette (82) (p. 9) also states that six or seven cells are budded into the interior of the embryo of *Stylochus* as upper endoderm, but does not give further information as to their exact origin.

In *Planocera inquilina*, on the other hand, the three anterior cells of the fourth quartet, viz., 4a, 4b, and 4c, do not divide at this time nor for a long time afterwards, if ever. The very large nuclei of these three cells can be traced up until just before the formation of the alimentary canal (fig. 36), and at this time they have not divided. The nuclei of these cells, especially 4b, become exceedingly large (fig. 32) and form excellent landmarks in the later stages.

Wilson (98) finds and figures the bilateral division of 4d. He states that the division of this cell into equal halves is an exception to the rule, and that in about 90 per cent. of the eggs of *Leptoplana* the division is markedly unequal (cf. his fig. 6, D, E and F). Wilson did not follow the future division of these cells. He says (p. 22): "As regards the fate of these cells, the inequality of 4d¹ and 4d² [4d^{1.1} and 4d^{1.2}] (often very marked) is itself indirect evidence that they do not give rise to symmetrical mesoblast bands as in the higher types, and I find no evidence that either of them gives rise to mesoblast cells. Both seem to have the same fate as the other entoblast cells, with which they exactly agree in deutoplasmic structure, and enter into the formation of the archenteron, as Lang has shown in the case of *Discocalis*."

It is peculiar that Lang should have observed cells budding into the interior of the egg from three cells of the fourth quartet and not from its other member, while I find that it is only this latter cell which divides towards the interior, or in fact the only cell of this quartet that divides at all. Lang gives figures of the spindles in the three anterior cells of the fourth quartet in *Discocalis* and also a detailed description of these divisions. Although this work was done on living eggs, it does not seem probable that so careful an observer would be mistaken in the facts. We must conclude, then, that the three upper endoderm cells of *Discocalis* are in fact endoderm, and that this species differs from *Planocera* in the division of the three anterior cells of the fourth quartet. We would not be surprised to find such a coenogenetic difference in different species of polyclads. *Discocalis* perhaps shows a more primitive condition in this respect, in that cells which are to

form part of the alimentary canal arise early from these three cells of the fourth quartet. In *Planocera* apparently most of the protoplasmic material has been separated from the cells 4a, 4b, and 4c in the previous divisions, and they now contain little more than a mass of yolk granules.

Lang, no doubt, overlooked the internal budding of the posterior cell, 4d, if such occurs in *Discocalis*. This internal division of 4d in *Planocera* is very evident and striking. In other particulars the cleavage of *Planocera inquilina* is so similar to that of other polyclads that it is difficult to believe that this species differs so fundamentally in respect to 4d. As stated before, practically all the previous work on polyclad embryology has been done on the living eggs alone. Such a division as the internal budding of 4d might easily be overlooked in the living opaque eggs. On this basis we might well conclude that in all probability such a division was overlooked by Lang and his predecessors.

Wilson, however, who undoubtedly was on the lookout for just such a division, did not find it in *Leptoplana*. Wilson says that he does not attempt to "describe the cleavage of *Leptoplana* in detail, but only indicate its leading features." I cannot but believe that in this statement is contained the reason why Wilson did not find mesoderm arising from 4d. A process so evident and significant as these divisions of 4d in *Planocera* can scarcely be conceived of as a coenogenetic character of one or even a few species of polyclads. In annelids and mollusks the bilateral division of 4d and the origin of mesoderm bands from these cells is without exception in the numerous species so far studied. Both Wilson and myself have now shown that the ectoderm of polyclads is segregated in the first three quartets of micromeres, and that the second quartet gives rise to some mesoderm. This process, so exactly paralleled in mollusks and annelids, can now scarcely be doubted as constant in its main features for all polyclads. Whether such a uniformity in the origin of the mesoderm from 4d will be found to hold throughout these Turbellaria can only be proven by further investigations on other species. I believe that certainly the weight of evidence is in favor of this uniformity.

The resemblance in the behavior of 4d in the polyclads to the homologous cell in annelids and mollusks becomes only more striking as we consider the details of its divisions. It is true that in annelids and mollusks 4d divides into two bilateral halves before it buds cells into the interior, while in *Planocera* 4d first buds a single cell into the segmentation cavity and then each of the two divides bilaterally (figs. 19, 25, 26). In either case exactly the same result is reached, and the delay of the bilateral cleavage for one-cell generation can certainly be very easily accounted for as a coenogenetic modification.

Conklin (97) was the first to point out that in the gasteropod *Crepidula* the cell $4d$ gave rise to both endoderm and mesoderm. In *Crepidula* four approximately equal cells are at first formed from $4d$. The two lower and external are pure entoblasts. Each of the two upper cells later gives off another entoblastic cell before they give rise to the mesoblast bands. Since that time numerous observers have found that in both annelids and mollusks the cell $4d$ is mesentoblastic. Wilson (98), in the paper so often referred to above, shows that a reinvestigation of *Nereis* proves that a number of small entoblast cells are budded off from the two halves of $4d$ before these form the mesodermal bands. He also shows that a series of stages may be found in different annelids and mollusks, ranging from a single pair of minute "vestigial" entoblast cells in *Aricia* and *Spio* to *Nereis* where from six to ten small cells are budded off, and to the condition in *Crepidula* where more than half the bulk of $4d$ forms endoderm. Around these and other facts Wilson has elaborated a beautiful theory of ancestral reminiscence. To this series, agreeing very closely with *Crepidula*, we may add the polyclad *Planocera inquilina*. Here, as in *Crepidula*, the two lower superficial cells derived from $4d$ are purely entoblastic and, as has been shown, give rise to very nearly all of the alimentary canal. Two more small cells, $4d^{2.1.1}$ and $4d^{2.2.1}$, derived from the two upper cells of $4d$, are probably added to the endoderm, while the remainder forms mesoderm. This close, almost astonishing, agreement of *Planocera* with annelids and mollusks cannot be without some significance. As Wilson (98) (p. 13) says: "If we accept Lang's view, which is supported by a large amount of evidence, that the platodes are not very far removed from the ancestral prototype of annelids and mollusks, we should expect to find in the polyclad a mode of cleavage to which that of the higher forms can in its main features be reduced."

Before Wilson's work this resemblance between polyclads and higher forms had seemed to be "only in the *form* of cleavage and not, so to speak, in its substance." I believe that now this difficulty has been entirely removed, and the polyclad cleavage not only conforms to the higher types in its "main features" but, as we have seen, in many of its details. These facts here set forth cannot but lend additional weight to the view already expressed on comparative anatomical grounds that the polyclads represent an offshoot from the same ancestral branch which later gave rise to the annelids and mollusks. On the other hand, it is a remarkable and interesting fact that phyla which must have separated from the common stock and from each other long ages ago still show such remarkable resemblances in their

early ontogenies. Yet I think this is the only interpretation we can put upon the facts. With our present knowledge of "Entwickelungs-mechanik" we cannot interpret these resemblances as due to external, mechanical conditions. Why the ectoblast should be segregated in three and only three quartets in polyclads, annelids, mollusks and, as Biglow (02) has shown, in some crustaceae, and why in these same groups mesoderm should arise from the posterior or left posterior cell of the fourth quartet and from no other of this quartet, are questions which do not readily lend themselves to a mechanical explanation under our present theories. We must for the present at least regard these resemblances as facts of heredity and hence of phylogentic value.

The origin of the alimentary canal in *Planocera* is unique among animals, so far as I am aware. *The whole of the alimentary canal arises from a portion of the posterior cell of the fourth quartet, while the other three cells of this quartet and all four of the macromeres are used as food or degenerate and give rise to no morphological structure. Not one of the last seven cells mentioned ever divides after its formation at the thirty-two-cell stage.* Surprising and unique as this phenomenon may be, it does not necessarily invalidate our present conception of the development of germ layers or their organs. Since the establishment of cell-lineage work it has become well known that many embryonic cells are formed in early stages which are destined never to divide again, nor to take any further part in the organization of the embryo. Compare for example the "turret" cells of certain mollusks as *Crepidula* (Conklin, 97). As has been pointed out, it is well known that in many annelids and mollusks the cell 4d gives rise to a portion of the alimentary canal. In these animals however the other three cells of this quartet as well as the macromeres take part in the formation of the digestive tract. Indeed these latter cells furnish the major portion of the alimentary tissue. With these facts in mind, we must regard the condition of the macromeres and the three anterior cells of the fourth quartet in *Planocera* as reminiscent of a time when all eight of these cells took part in the formation of the alimentary tract. Thus the embryology shows that this worm is specialized in this respect and must long ago have left the track which led on to annelids and mollusks.

This peculiar development of the alimentary canal in the polyclads offers the suggestion that it may be a step toward the development of such forms as the acoelous rhabdocoeles, in which the alimentary canal is altogether absent. But the embryology of these as well as other turbellaria present such great variations from the type found in the polyclads that it is useless to speculate along this line.

SUMMARY.

The cleavage of the eggs of *Planocera inquilina* Wh. until a late stage (forty-four cells) is strictly spiral in the dextral sequence.

Three quartets of ectomeres are given off in alternating dextrotropic and læotropic directions. At the next division a fourth quartet is formed, the cells of which are of very large size and contain most of the yolk. The "macromeres" are very minute cells which remain at the vegetative pole until the closure of the blastopore. The marked degenerative character of their nuclei and the small amount of cytoplasm indicate that they degenerate without giving rise to any structure (p. 529).

At the stage with forty cells there are formed at the animal pole four small "apical" cells, which in their method and time of origin correspond closely to the cells of the same name in annelids and mollusks (p. 530).

At the forty-four-cell stage the posterior cell of the fourth quartet, 4*d*, buds a single large cell into the interior of the embryo. Both of these cells, 4*d*¹ and 4*d*², next divide bilaterally (p. 536).

Of these four cells the two upper and inner give rise to a portion of the mesoderm and possibly a small part of the endoderm (p. 537). The lower pair of cells, lying on the surface of the embryo, give rise to practically all of the endodermal part of the alimentary canal (p. 541). Thus the history of this cell, 4*d*, shows a remarkable resemblance to its homologue in mollusks and annelids.

The three anterior cells of the fourth quartet, 4*a*, 4*b* and 4*c*, seem to function only as the bearers of food yolk and apparently give rise to no morphological structure. The very large nuclei of these cells can be followed until the beginning of the pharyngeal invagination. The yolk in these cells breaks up into spherules, probably through the action of enzymes from the large nuclei. This liquified yolk is later absorbed by the endoderm cells (p. 542).

A large portion of the ectoderm is formed by the successive budding or delimitation of small cells from larger, deeper lying ones (p. 533).

A portion of the mesoderm, chiefly that part lying around the pharynx, is derived from cells of the second quartet, and thus corresponds with the "secondary" mesoblast or "larval" mesenchyme of annelids and mollusks (p. 535).

In the spiral cleavage, the segregation of the ectoblast in three quartets, the formation of a large part of the mesoderm from 4*d*, the formation of the apical cells, and in many other details the development

of these platodes corresponds to that of annelids and mollusks. These facts must tend to confirm the view that in their early history these platodes were closely related to the two last mentioned phyla.

On the other hand, in the development of the entire alimentary canal from a portion of the mesentoblast, 4*d*, and in the consequent degeneration of the "macromeres" and of the remaining cells of the fourth quartet, this polyclad is unique. This peculiar development of the alimentary tract shows that the cleavage of the polyclads, while closely resembling that of other groups, is not a generalized type.

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EXPLANATION OF PLATES XXXV-XL.

All figures of fixed and stained eggs, except fig. 35, were drawn with Zeiss Camera lucida at table level under Leitz obj. $\frac{1}{2}$; oc. 2. Figures of living eggs (viz. 1, 2, 3, 4, 6, 8, 10 and 11) were drawn as above, but with Leitz obj. 7; oc. 2. Fig. 35 was drawn with B and L. obj. $\frac{1}{2}$; oc. 1. All drawings have been reduced one-third.

REFERENCE LETTERS.

<i>e.</i> , entoblasts $4d^{2.1.1}$ and $4d^{2.2.1}$.	<i>n.4b.</i> , nucleus of the cell 4b.
<i>end.</i> , endoderm.	<i>ph.</i> , pharynx.
<i>ey.</i> , eye.	<i>r.</i> , rhabdites.
<i>g.</i> , ganglion.	<i>y.</i> , yolk.
<i>mes.</i> , mesoderm.	

PLATE XXXV.—Fig. 1.—Living egg before first maturation division, showing large germinal vesicle.

Fig. 2.—Living egg during the second maturation division, showing amœboid processes.

Fig. 3.—Living egg after second maturation division.

Fig. 4.—Living egg during the first division. The cell *C-D* is slightly larger than *A-B*.

Fig. 5.—Stained egg in the second division. From left side, showing the crossing of the spindles.

Fig. 6.—Living egg in the four-cell stage, from the animal pole.

Fig. 7.—Formation of the first quartet. From right side, showing dextrotropic cleavage.

Fig. 8.—Living egg in the eight-cell stage. From animal pole.

PLATE XXXVI.—Fig. 9.—Læotropic division of the preceding eight cells. The spindles in the *D* quadrant show an advance over the others. From the animal pole.

Fig. 10.—Living egg with sixteen cells. From the animal pole.

Fig. 11.—Same as fig. 10, but from vegetative pole.

Fig. 12.—Dextrotropic divisions of cells $1a^1-1d^1$ and of $2a-2d$. From animal pole.

Fig. 13.—Thirty-two cells. Dextrotropic divisions of $1a^{2.1}-1d^{2.1}$. From animal pole.

Fig. 14.—Thirty-two cells, showing dextrotropic formation of $3a$ and $3c$. Vegetative pole.

Fig. 15.—Thirty-two cells from vegetative pole, showing spindles for formation of $4b$ and $4d$.

PLATE XXXVII.—Fig. 16.—Same as Fig. 15, from right side.

Fig. 17.—Thirty-six cells from near vegetative pole. Spindle in $2c^2$. $2a^2$ and $2d^2$ have divided.

Fig. 18.—Thirty-nine cells. From animal pole, showing læotropic formation of apical cells.

Fig. 19.—Forty-five cells, from the right posterior side. Shows the first division of the mesentoblast $4d$. $2d^1$ also dividing.

Fig. 20.—Forty-seven cells. From vegetative pole.

Fig. 21.—About the same stage as fig. 20, drawn from the right side, shows the unequal division of $2c^1$ and $2b^1$. Also spindles in $1c^{2.1}$ and $1b^{2.1}$. $1a^{1.2}-1d^{1.2}$ have divided dextrotropically (see fig. 22).

PLATE XXXVIII.—Fig. 22.—Slightly older egg than fig. 21, drawn from left upper side. Shows division of $1a^{1.1.2}-1d^{1.1.2}$ and of $1b^{1.2}$. $1a^{1.2}$ and $1b^{1.2}$ have divided previously. Also shows læotropic division of $1a^{2.1}-1d^{2.1}$.

Fig. 23.—Still older egg, showing the same division as fig. 22, but more advanced. From animal pole.

Fig. 24.—Egg from animal pole, showing preceding divisions completed and dextrotropic division of $1d^{2.2}$. Also the bilateral division of $4d^2$.

Fig. 25.—Similar stage from vegetative pole. Shows spindle for bilateral division of $4d^2$. Also læotropic division of $2a^{2.1}-2d^{2.1}$. $3d$ preparing to divide.

Fig. 26.—Shows division of $4d^2$ completed and spindle in $4d^1$. Also shows division of $2d^2$ and $2b^2$, of $3d$ and $3b$, and of $2a^{2.1}$.

Fig. 27.—Similar stage from the left side. Shows division of $2a^{2.1}$, $4d^1$ and $2b^{2.2}$; also of $1a^{1.2.1}$.

PLATE XXXIX.—Fig. 28.—Egg from animal pole, showing divisions of $1a^{1.1.2.2}-1d^{1.1.2.2}$; also of $1a^{1.2.1}$ and $1c^{1.2.1}$.

Fig. 29.—Similar view of slightly older egg, showing preceding divisions completed and the resulting small superficial cells $1a^{1.1.2.2.2}-1d^{1.1.2.2.2}$.

Fig. 30.—Egg from near the vegetative pole, showing division of $4d^1$ completed and budding in of the cells $2b^{1.1}$ and $2d^{1.1}$.

Fig. 31.—Egg from animal pole at slightly later stage than fig. 29. Shows divisions of $4d^{2.1}$ and $4d^{2.2}$. The resulting small cells $4d^{2.1.1}$ and $4d^{2.2.1}$ are probably entoblasts.

Fig. 32.—Optical section from animal pole, showing the cells $2a^{1.1}-2d^{1.1}$ budding mesoderm to the interior. Also shows the large nuclei of $4b$, $4a$ and $4c$.

Fig. 33.—Later stage, showing the internal divisions of the entoblasts $4d^1$ and $4d^2$. Not all the cells on the interior of the egg are shown.

PLATE XL.—Fig. 34.—Optical section of egg from the posterior side. The entoblasts $4d^{1.1}$ and $4d^{1.2}$ have divided once and possibly twice. The two small entoblasts $4d^{2.1.1}$ and $4d^{2.2.1}$ are shown with dark nuclei. Above these are the two small mesoblasts, while the larger mesoblasts $4d^{2.1.2.2}$ and $4d^{2.2.2.2}$ are again dividing. Above these are a number of cells from the first quartet which later form the ganglion. The large nuclei of $4a$, $4b$ and $4c$ are shown in dotted outlines.

Fig. 35.—Optical section of an egg viewed from near the vegetative pole. The derivatives of $4d^2$ are stippled. The division of $4d^{2.1.2.1}$ and $4d^{2.2.2.2}$ indicated in fig. 34 are now completed and the beginning of the mesodermal bands is evident. In many eggs the divisions are not so regular as shown here. Instead the mesoderm tends to form clusters of cells rather than bands.

Fig. 36.—Optical section of a much later stage. From the left side. The mass of endoderm derived from $4d^1$ lies just above the future pharynx. The mesoderm cells have passed to the periphery and the centre of the egg is filled with the homogeneous yolk spheres (y), in which the shrunken nucleus of $4b$ can be seen ($n.4b.$). The two small cells marked (e) are probably the entoblast $4d^{2.1.1}$ and $4d^{2.2.2}$.

Fig. 37.—Optical section of an older embryo. From the posterior side. Shows ectodermal pharynx and beginning of lumen in the endoderm.

Fig. 38.—Actual longitudinal section of a still older embryo, showing the backward turn of the alimentary canal in this stage.

Fig. 39.—Longitudinal section of a Muller's larva. Nearly all the yolk spheres have disappeared by this time and the alimentary canal is greatly enlarged but still unbranched.